DAC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	09/316,935	Confirmation No. 8495
Applicants	:	Cornelis J.M. Melief et al.	
Filed	:	May 22, 1995	
Title	:	CD-40 BINDING MOLECULES AND CTL PEPTIDES FOR TREATING TUMORS	
Art Unit	:	1632	
Customer No.	:	28289	

#24

PETITION TO REVIVE UNDER 37 C.F.R. § 1.137(b)

MAIL STOP PETITION
Commissioner for Patents
Washington, DC 20231

Sir:

The Applicants hereby submit this Petition to Revive the above-captioned patent application. The entire delay in filing the required reply from the due date for reply until the filing of a grantable petition pursuant to 37 C.F.R. § 1.137(b) was unintentional.

This Petition is accompanied by the following:

(1) A Petition fee set forth under 37 C.F.R. § 1.17(m) of $1500.00; and

(2) A Reply in the form of continuation application United States Application No. 10/227,789.

No Terminal Disclaimer and fee is required under 37 C.F.R. § 1.20(d), as this utility application was filed after June 8, 1995.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on June 27, 2006.

Linda L. Marlowe
(Name of Person Mailing Paper)

Linda L. Marlowe 6/27/2006
Signature Date

STATEMENTS

The above-identified application (U.S. Patent Application No. 09/316,935; hereinafter referred to as "the '935 parent application") became abandoned for failure to reply within the meaning of 37 C.F.R. § 1.113 in a timely manner to the final Office Action mailed October 10, 2001, which set a shortened statutory period for reply of three (3) months. An Amendment and a two-month extension of time were filed on March 12, 2002. Accordingly, the application became abandoned on March 13, 2002.

The Applicants filed a Petition to Revive under 37 C.F.R. § 1.137(b) for unintentional abandonment on August 26, 2002, submitting therewith a continuation application as the required reply, now U.S. Patent Application No. 10/115,620.

On the same day, August 26, 2002, that the Petition to Revive was submitted to revive the '935 parent application, Applicants filed a continuation application to the '935 parent application, now U.S. Patent Application No. 10/227,789 (hereinafter referred to as "the '789 application") in the United States Patent and Trademark Office.

The decision granting the Petition to Revive was issued on September 16, 2002. In the decision granting the Petition, it was stated that "[t]his application is being revived solely for purposes of continuity. As continuity has been established by this decision, the application is again abandoned in favor of continuing application No. **10/115,620.**" (U.S. Patent Application No. 10/115,620 hereinafter will be referred to as "the '620 application").

Applicants respectfully submit that the grant of the Petition to revive the '935 parent application to provide continuity to the '620 application, filed April 4, 2002, incorrectly omitted continuity to any other continuation application(s) to the '935 parent application filed on or before September 16, 2003, such as the '789 application, filed August 26, 2002.

Applicants received an Office Action, dated January 27, 2006, in the '789 application, in which it is stated, on page 3, that "Applicant has not complied with one or more conditions for domestic priority under 35 USC 120..." The Office Action states that "the 09/316,935 application was abandoned on 3/13/02. As such, parent application 09/316,935 was not copending with the instant application, which was filed on 8/26/02."

Applicants respectfully request revival of the '935 parent application to provide continuity to the '789 application in order to claim priority to the '935 parent application, which continuity Applicants submit was incorrectly omitted when the Petition to Revive was granted for the '935 parent application on September 16, 2002.

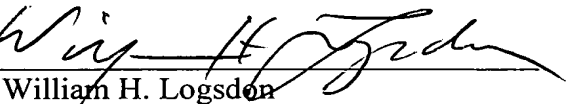
The '789 application, which is a continuation application of the '935 application, is hereby submitted as the required reply.

Applicants can no longer claim small entity status. A check in the amount of $1500.00 is hereby submitted to cover the fee for unintentional delay. If further fees are necessary, the Commissioner is hereby authorized to charge any additional fees which may be required to Deposit Account No. 23-0650. Please refund any overpayment to Deposit Account No. 23-0650. The original and a copy of this submission are enclosed.

The Examiner's favorable decision is respectfully requested.

Respectfully submitted,

THE WEBB LAW FIRM

By 

William H. Logsdon
Registration No. 22,132
Attorney for Applicants
700 Koppers Building
436 Seventh Avenue
Pittsburgh, PA 15219
Telephone: (412) 471-8815
Facsimile: (412) 471-4094
E-Mail: webblaw@webblaw.com

PTO/SB/05 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Please type a plus sign (+) inside this box → ⊞

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	TNX98-04-02
First Inventor	Melief et al
Title	CD40 Binding molecules and CTL Peptides for Treating Tumors
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [xx] Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. [xx] Applicant claims small entity status. See 37 CFR 1.27.
3. [xx] Specification [*Total Pages* 22] *(preferred arrangement set forth below)*
   - Descriptive title of the invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. [xx] Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 8]
5. Oath or Declaration [*Total Pages* 3]
   - a. [ ] Newly executed (original or copy)
   - b. [xx] Copy from a prior application (37 CFR 1.63 (d)) *(for continuation/divisional with Box 18 completed)*
     - i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. [ ] Application Data Sheet. See 37 CFR 1.76

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

7. [ ] CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   - a. [ ] Computer Readable Form (CRF)
   - b. Specification Sequence Listing on:
     - i. [ ] CD-ROM or CD-R (2 copies); or
     - ii. [xx] paper
   - c. [xx] Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. [ ] Assignment Papers (cover sheet & document(s))
10. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
11. [ ] English Translation Document *(if applicable)*
12. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
13. [ ] Preliminary Amendment
14. [xx] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [ ] Nonpublication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. [ ] Other: .....

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:*

[xx] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No.: 09 / 316,935

Prior application information: *Examiner* A. Beckerleg *Group Art Unit:* 1632

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS

[xx] *Customer Number or Bar Code Label* 22689 *(Insert Customer No. or Attach Bar Code Label Here)* or [ ] *Correspondence address below*

Name					
Address					
City		*State*		*Zip Code*	
Country		*Telephone*		*Fax*	

Name (Print/Type)	Cheryl A. Liljestrand	*Registration No. (Attorney/Agent)*	45,275
Signature	Cheryl A. Liljestrand	*Date*	August 26, 2002

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.

PTO/SB/17 (11-00)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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FEE TRANSMITTAL for FY 2001

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT ($) 510.00

Complete if Known	
Application Number	TO BE ASSIGNED
Filing Date	August 26, 2002
First Named Inventor	MELIEF, Cornelius et al
Examiner Name	TO BE ASSIGNED
Group Art Unit	1632
Attorney Docket No.	TNX98-04-02

RECEIVED
JUL 06 2006
OFFICE OF PETITIONS

METHOD OF PAYMENT

1. [X] The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
   - Deposit Account Number: 20-0087
   - Deposit Account Name: Tanox, Inc.
   - [XX] Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
   - [XX] Applicant claims small entity status. See 37 CFR 1.27
2. [ ] **Payment Enclosed:** [ ] Check [ ] Credit card [ ] Money Order [ ] Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	710	201	355	Utility filing fee	370.00
106	320	206	160	Design filing fee	
107	490	207	245	Plant filing fee	
108	710	208	355	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below	Fee Paid
Total Claims	17	-20** =	–	X	=
Independent Claims	3	- 3** =	0	X	=
Multiple Dependent				140	= 140

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	80	209	40	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 510.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	390	216	195	Extension for reply within second month	
117	890	217	445	Extension for reply within third month	
118	1,390	218	695	Extension for reply within fourth month	
128	1,890	228	945	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,240	241	620	Petition to revive - unintentional	
142	1,240	242	620	Utility issue fee (or reissue)	
143	440	243	220	Design issue fee	
144	600	244	300	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	710	246	355	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	710	249	355	For each additional invention to be examined (37 CFR § 1.129(b))	
179	710	279	355	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Cheryl A. Liljestrand	Registration No. (Attorney/Agent)	45,275	Telephone	713-578-4182
Signature	Cheryl A. Liljestrand			Date	August 26, 2002

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney's Docket 98-4

As a below named inventor, I hereby declare that:

This declaration is for an original application.

My residence, post office address and citizenship are as stated below next to my name. I believe I am an original, joint and first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"CD-40 Binding Molecules and CTL Peptides for Treating Tumors"

the specification of which was filed under Serial No. 09/316,935 on 5/22/99.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which I know to be material to the patentability of this application as defined in Title 37, Code of Federal Regulations Section 1.56.

As a named inventor, I hereby appoint the following attorney to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Eric P. Mirabel
Reg. No. 31,211

Send correspondence and direct telephone calls to:

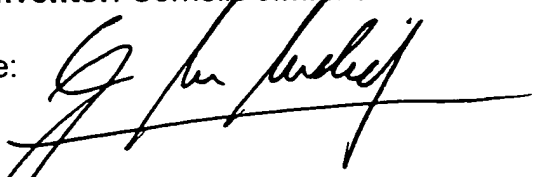
Eric Mirabel
Tanox, Inc.
10301 Stella Link #110
Houston, TX 77025-5497
(713) 664-2288

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the Office information which I know to be material to patentability as defined in Title 37, Code of Federal Regulations, _ 1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

US Provisional Patent Application No. 60/086,625 filed 5/23/98.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of **first inventor**: Cornelis J.M. Melief

Inventor's signature: 

Date: 10/11/99 Country of Citizenship: The Netherlands
Residence and Post Office Address: Wilhelminapark 33, 2012 KC Haarlem, The Netherlands

Full name of **second inventor**: Stephen P. Schoenberger,

Inventor's signature: 

Date: Nov. 18, 1999 Country of Citizenship: USA
Residence and Post Office Address: 259 Coneflower Street, Encinitas CA 92024

Full name of **third inventor**: Rienk Offringa

Inventor's signature:

Date: NOV. 10, 1999 Country of Citizenship: The Netherlands
Residence and Post Office Address: Stieltjesstraat 63, 2313 SJ Leiden, The Netherlands

Full name of **fourth inventor**: Rene Toes

Inventor's signature: Rene Toes

Date: NOV. 10, 1999 Country of Citizenship: The Netherlands
Residence and Post Office Address: Hogewoerd 32c, 2311 HN Leiden, The Netherlands

PATENT
ATTORNEY DOCKET NO.: TNX 98-04-02
CUSTOMER NO: 26839

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:
MELIEF, Cornelis, et al.

Serial No.: TO BE ASSIGNED

Filed: AUGUST 26, 2002

For: CD40 BINDING MOLECULES AND CTL PEPTIDES FOR TREATING TUMORS

Group Art Unit: TO BE ASSIGNED

Examiner: TO BE ASSIGNED

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

REQUEST TO USE CRF FROM PRIOR APPLICATION

In compliance with the Sequence Listing requirements of 37 C.F.R. §1.821 (b) through (f), Applicants hereby submit a Sequence Listing. The paper copy of the Sequence Listing in this application is identical to the computer readable copy of the Sequence Listing filed in application 09/316,935 filed May 22, 1999, a corrected Sequence Listing and Substitute CRF having been filed on April 3, 2001. In accordance with 37 CFR 1.821(e), please use the Substitute computer readable form (CRF) filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the instant application. A paper copy of the Sequence Listing is included herewith.

Respectfully Submitted,

Dated: August 26, 2002.

BY: Cheryl A. Liljestrand
Cheryl A. Liljestrand
Reg. No. 45,275

SEQUENCE LISTING

<110> MELIEF, Cornelius
Schoenberger, Stephen
Offringa, Rienk
Toes, Rene

<120> CD-40 Binding Molecules and CTL Peptides for Treating Tumors

<130> TNX 98-04

<140> 09/316,935
<141> 1999-05-22

<150> US PROVISIONAL 60/086,625
<151> 1998-05-23

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1 5 10

<210> 3

<211> 9

<212> PRT

<213> Human Papilloma Virus Type 16 E7 derived peptide

<400> 3

Arg Ala His Tyr Asn Ile Val Thr Phe
1 5

PATENT
ATTORNEY DOCKET NO.: TNX 98-04-02
CUSTOMER NO: 26839

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:
MELIEF, Cornelis, et al.

Serial No.: TO BE ASSIGNED

Filed: AUGUST 26, 2002

For: CD40 BINDING MOLECULES AND CTL PEPTIDES FOR TREATING TUMORS

Group Art Unit: TO BE ASSIGNED

Examiner: TO BE ASSIGNED

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97(b)

Pursuant to 37 C.F.R. §§ 1.56 and 1.97(b), applicants submit the documents listed on the attached PTO 1449. This Information Disclosure Statement is being filed within three months of the filing date of the above-referenced application.

Copies of the listed documents were submitted in the parent application, Serial No. 09/316,935. Applicants respectfully request that the Examiner consider the listed documents and indicate that they were considered by making appropriate notations on the attached PTO 1449 form.

This submission does not represent that a search has been made or that no other documents exist which may be material to the examination of this application and does not constitute an admission that any of the listed documents, or any part thereof, are material or constitute prior art under title 35 of the United States code. If the Examiner applies any of the documents as prior art against any claim in the application,

Applicants reserve the right to present to the Office the relevant facts and law regarding the appropriate status of such documents should the Applicant(s) determine(s) that the cited document(s) do not constitute "prior art".

Applicant further reserves the right to take appropriate action to establish the patentability of the disclosed invention over the listed documents, should one or more of the documents be applied against the claims of the present application.

Respectfully Submitted,

Dated: August 26, 2002

BY: Cheryl A. Liljestrand
Cheryl A. Liljestrand
Reg. No. 45,275

Please type a plus sign (+) inside this box [+]

PTO/SB/08A (08-00)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

SHEET 1 OF 2

Complete if Known	
Application Number	**TO BE ASSIGNED**
Filing Date	**August 26, 2002**
First Named Inventor	**MELIEF, ET AL**
Group Art Unit	**TO BE ASSIGNED**
Examiner Name	**TO BE ASSIGNED**
Attorney Docket Number	**TNX98-04-02**

U.S. PATENT DOCUMENTS

Examiner Initials	U.S. Documents Number	Kind Code (if known)	U.S. Patent Document Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	5874082		DE BOER	02-23-1999	

FOREIGN DOCUMENTS

Examiner Initials	Foreign Patent Documents Office	Number	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation
	PCT	97/41440	A1	VAN DER BURG ET AL.	11-06-1997		N/A

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Please type a plus sign (+) inside this box [+]

PTO/SB/08A (08-00)
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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary) SHEET 2 OF 2	*Complete if Known*	
	Application Number	**TO BE ASSIGNED**
	Filing Date	**August 26, 2002**
	First Named Inventor	**MELIEF, ET AL**
	Group Art Unit	**TO BE ASSIGNED**
	Examiner Name	**TO BE ASSIGNED**
	Attorney Docket Number	**TNX98-04-02**

OTHER PRIOR ART -- NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s),	T2
	C1	Lipford, G. B., et al.; "Peptide Engineering Allows Cytotoxic T-Cell Vaccination Against Human Papiloma Virus Tumour Antigen, E6", *Immunology* 84:298-303 (1995).	
	C2	Christ, M., et al., "Gene Therapy With Recombinant Adenovirus Vectors: Evaluation of the Host Immune Response", *Immunology Letters*, 57:19-25 (1997).	
	C3	Naviaux, R. et al.; "Retroviral Vectors For Persistent Expression *In Vivo*", *Current Opinion in Biotechnology* 3:540-547 (1992).	
	C4	Gunzburg, W. H. et al.; "Virus Vector Design In Gene Therapy", *Molecular medicine today*,Vol. 1(9), pp. 410-417 (1995).	
	C5	Urvanelli, D., et al., "C-Terminal Domain Of The Adenovirus E1A Oncogene Product Is Required For Induction of Cytotoxic T Lymphocytes And Tumor-Specific Transplantation Immunity", Virology 173:607-614 (1989).	
	C6	Funakoshi, D., et al.; "Inhibition Of Human B-Cell Lymphoma Growth By CD40 Stimulation, *Blood*, Vol. 83(10), pp. 2797-2794 (1994).	
	C7	Tong, Alex. W. Et Al; "Anti-CD40 Antibody Binding Modulates Human Multiple Myeloma Clonogenicity In Vitro", Blood, Vol. 84(9), pp. 3026-3033 (1994).	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

CD40 BINDING MOLECULES AND CTL PEPTIDES FOR TREATING TUMORS

Field of the Invention

The invention includes CD40 binding molecules together with CTL-activating peptides, including tumor antigens, in a pharmaceutical composition.

Background of the invention

Many tumors escape surveillance by our immune system. In cancer patients there is clearly a quantitative and/or qualitative defect in the immune system's specific mechanisms to delete tumor cells. One of these mechanisms is provided by the cytotoxic T cells (CTL) that can recognise and kill cells infected by virus or transformed into cancer cells. Previously it was postulated that dendritic cells (DC) stimulate T-helper cells which, in turn, provide help for the activation of CTL. The present inventors have demonstrated that the T-helper cell is not providing helper signals directly to the CTL (by secretion of IL2), but rather, the T-helper cell is providing a signal to the DC that induces yet uncharacterised cell surface and/or soluble molecules that can activate CTL in the absence of T-helper cells. The signal provided by the T-helper cell to the DC is mediated by CD40L-CD40 interaction. This novel finding has provided an unique opportunity for cancer immunotherapy.

The immune system is capable of killing autologous cells when they become infected by virus or when they transform into cancer cells. Such a potentially dangerous mechanism must, clearly, be under tight control. The immune system's CTL circulate as

inactive precursors. To be activated, the precursor T-killer cell must recognise its specific antigen peptide, which is presented as MHC class I molecules on professional APC. However, in order to prime these T cells, the APC also need to present the antigen in a proper costimulatory context as provided by, amongst others, the costimulatory surface molecules B7.1 and B7.2 and by the lympokine IL-12.

Until recently it was believed that a T-helper cell that recognises the same antigen on the same APC is needed to fully activate the CTL. The specific T-helper cell would supply cytokines such as IL-2 needed for the activation of the CTL. Guerder and Matzinger (J. Exp. Med. 176:553 (1992)), however, proposed the "licensing" model for CTL activation. In this model it was suggested that the T-helper cell, when recognising its antigen on a professional APC, would deliver an activation signal to the APC that as a result would be able to subsequently activate a CTL without the need for the T-helper cell to be present. Only very recently, the molecular mechanism of the licensing model has been elucidated. Schoenberger et al. (Nature 393:480 (1998)), described the role of the CD40L-CD40 pathway in the licensing model. Interaction between T-helper cell and DC through the CD40-CD40L binding results in activation of the DC, thereby enabling the DC to efficiently prime naive CTL.

DC circulate through the tissues of our body and in this manner can collect, process and present antigens. After collection of antigens, they migrate to the draining lymph nodes where they present antigen to the T cells. It is well known that a DC needs to be activated to perform optimally. Resting DC express only modest levels of MHC and costimulatory molecules and are poor stimulators of T cells. DC can be activated by

inflammatory cytokines and bacterial products, which results in upregulation of MHC and costimulatory molecules. Activation of DC into fully mature DC, expressing optimal levels of MHC molecules, costimulatory molecules and lymphokines such as IL-12, requires additional triggering of these cells through the CD40 receptor. Consequently, the combination of inflammatory cytokines at the site of antigen uptake and the CD40L-CD40 interaction during the T-helper cell interaction result in an optimal capacity to license the DC for CTL activation.

Many tumors escape immune surveillance by specific CTL mechanisms. If DC gather tumor antigens under non-inflammatory conditions the number of T-helper cells that are activated may be to low to induce enough CTL to be activated to induce an appropriate anti-tumor response. This concept has prompted investigators to help the immune system by administration of cytokines such as IL-2 and IL-12 that directly stimulate CTL activity or by boosting antigen presentation by administration of tumor cells transfected with GM-CSF. These strategies have met variable but encouraging results.

It is clear that there is still a great need to find ways to generate and/or enhance protective anti-tumor responses involving cellular and humoral immunity. The CD40 activation pathway was found to be a major immunoregulatory pathway for the generation of primary humoral and cellular immune responses. As described above, the CD40 pathway on DC is responsible for the induction of anti-tumor CTL responses. In addition, activation of the CD40 pathway on macrophages stimulates a strong tumoricidal activity.

Summary of the Invention

The invention includes CD40 binding molecules together with CTL-activating peptides, including tumor antigens, in a pharmaceutical composition. Such composition is useful for enhancing the anti-tumor effect of a peptide tumor vaccine, or for otherwise activating CTLs so that the activated CTLs can act against tumorous or infected cells. The CD40 binding molecules can include antibody molecules, as well as homologues, analogues and modified or derived forms thereof, including immunoglobulin fragments like Fab, $(Fab')_2$ and Fv, as well as small molecules including peptides, oligonucleotides, peptidomimetics and organic compounds which bind to CD40 and activate the CTL response. CTL-activating peptides include the adenovirus-derived E1A peptide, having the sequence SGPSNTPPEI (SEQ ID NO:2), and the HPV16 E7 peptide derived from human papillomavirus type 16, having the sequence RAHYNIVTF (SEQ ID NO:3).

The CD40 binding molecule and the CTL activating peptide can be administered to a patient by suitable means, including injection, or gene constructs encoding such a molecule and a peptide can be administered, and the molecule and peptide thereby produced *in vivo* or *ex vivo*. Such a gene therapy is conducted according to methods well known in the art. If the transfection or infection of the gene constructs is done *ex vivo*, the infected or transfected cells can be administered to the patient, or these steps can be done *in vivo* whereby the molecule and the peptide are produced endogenously. The transfection or infection, if done *ex vivo*, can be by conventional methods, including electroporation, calcium phosphate transfection, micro-injection or by incorporating the gene constructs into suitable liposomes. Vectors, including a retrovirus, adenovirus or a

parvovirus vector, or plasmids, can be used to incorporate the gene constructs, which are then expressed *in vivo* or *ex vivo.*

It is demonstrated herein that T-cell help for CTL priming is mediated through CD40-CD40Ligand (CD40L) interactions, and that lack of CTL priming in the absence of $CD4^+$ T cells can be restored by monoclonal antibody (mAb)-mediated CD40 activation of bone marrow-derived APC in the presence of CTL-activating peptides including tumor antigens. Furthermore, blockade of CD40L, expressed by $CD4^+$ T cells, results in the failure to raise CTL immunity. This defect can be overcome by *in vivo* CD40-triggering. I*n vivo* triggering of CD40 can markedly enhance the efficacy of peptide-based anti-tumor vaccines, or otherwise activate CTLs to result in an anti-tumor or anti-infected cell reaction.

It is also noted that a CTL-activating peptide can become tolerogenic, meaning that the host reaction against cells expressing such peptide is inhibited, in the absence of anti-CD40. However, such a peptide combined with an activating anti-CD40 antibody converts tolerization into strong CTL activation. Moreover, as noted above, CD40-ligation can provide an already protective tumor-specific peptide-vaccine with the capacity to induce therapeutic CTL immunity in tumor-bearing mice.

These findings together demonstrate that the CD40-CD40Ligand pair acts as a switch determining whether naive peripheral CTL are primed or tolerized, Therefore CD40-binding agents such as monoclonal antibodies and other stimulatory ligands can be effectively used in combination with a CTL-activating peptide.

Brief description of the Figures

Figure 1: Cross-priming of E1B-specific CTLs requires $CD4^+$ T helper cells

Splenocytes from normal (*a*) or CD4-depleted B6 (*b*) mice immunized with Ad5E1-BALB/c MECs were tested at various effector/target ratios for lysis of syngeneic MEC target cells loaded with the $E1B_{192-200}$ peptide (solid lines), which is derived from human adenovirus and has the sequence VNIRNCCYI (SEQ ID NO:1)or a D^d-restricted control peptide HPV-16 $E7_{49-57}$ (dashed lines). Each line represents one mouse. Data shown represent one experiment of three performed with similar results.

Figure 2: CD40 activation replaces $CD4^+$ T helper cells

Splenocytes from CD4-depleted (*a, b*) or classII-deficient I-Ab-knockout (KO) (*c,d*) B6 mice were immunized with Ad5E1-BALB/c MECs and treated with either the CD40-activating antibody (Ab) FGK45 (*a, c*) or an isotype control antibody (*b, d*). These splenocytes were tested for E1B-specific CTL activity on syngeneic MEC target cells loaded with either the $E1B_{192-200}$ peptide (solid lines) or the HPV-16 $E7_{49-57}$ control peptide (dashed lines). Each line represents a single mouse. Data depicted are from two independent experiments. E/T ratio, effector/target ratio.

Figure 3: B cells are not essential as cross-priming APCs or for anti-CD40-mediated restoration of cross-priming

Spenocytes were taken from untreated (*a*), CD4-depleted B-cell-deficient B6 □MT mice (*b*, *c*), which were immunized with Ad5E1-BALB/c MECs and which received either an isotype control antibody (*b*) or the CD40-activating antibody FGK45 (*c*). These splenocytes were tested for E1B-specific CTL activity on syngeneic MEC target cells loaded with either the $E1B_{192-200}$ peptide (solid lines) or the HPV $E7_{49-57}$

control peptide (dashed lines). Each line represents one mouse. Data shown represent one experiment of two performed with similar results.

Figure 4: CD40L blockade prevents cross-priming of E1B-specific CTLs

Splenocytes were taken from B6 mice immunized with Ad5E1-BALB/c MECs and treated with the CD40L-blocking antibody MR-1 (***a***), or control antibody (***b***), or from mice treated with the CD40L-blocking antibody MR-1 in combination with the CD40-activating antibody FGK45 (*c*) 24h after immunization. These splenocytes were tested for E1B-specific CTL activity on syngeneic MEC target cells loaded with the $E1B_{192-200}$ peptide (solid lines) or the HPV-16 $E7_{49-57}$ control peptide (dashed lines). Each line represents one mouse. Data shown represent one experiment of three performed with similar results. E/T ratio, effector/target ratio.

Figure 5: Mice injected s.c. with the E1A-peptide are no longer able to mount E1A-specific CTL

C57BL/6 mice were injected twice s.c. (1 week interval) with 20 μg E1A-peptide (***a***, ***b***) or control-peptide (***c***, ***d***) in IFA, and challenged i.p. 1 day later with SAMB7 (***b***, ***d***), a cell line expressing high amounts of E1A-peptide. Bulk cultures derived from these mice were tested for E1A-specific cytotoxicity on target cells pulsed with the E1A-peptide (-■-) or the HPV16 E7-peptide (-O-). Specific lysis of representative bulk cultures at different effector to target (E/T) ratios is shown. This experiment has been repeated 4 times with comparable results.

Figure 6: Tolerizing E1A-peptide is rapidly distributed systemically after s.c. injection in IFA

Spleen cells derived from untreated C57BL/6 mice *(-)*, or from mice injected s.c. 16 h earlier with 100 µg of E1A- or HPV16 E7-peptide in IFA were used as stimulator cells for an E1A-specific CTL clone. [^{3}H]-thymidine incorporation (cpm) +/- *S.E.M.* is shown for different effector to stimulator concentrations, without subtraction of background counts. Results are representative of 5 independent experiments.

Figure 7: CTL-tolerance induction is reverted into CTL-priming after CD40-triggering *in vivo*

Wild type C57BL/6 mice (***a***, ***b***) and H-2^b CD40 $^{-/-}$ mice (***c***, ***d***) were injected s.c. with 20 µg E1A-peptide in IFA alone *(**c**)*, in combination with a rat IgG2a control antibody *(**a**)* , or in combination with the anti-CD40 mAb FGK-45 (***b***, ***d***). Bulk cultures from these mice were tested for E1A-specific cytotoxicity on target cells pulsed with the E1A-peptide (-■-), the HPV16 E7-peptide (-O-) or Ad5E1 transformed tumor cells (-◆-). Specific lysis of representative bulk cultures at different E/T ratios is shown. This experiment has been repeated 18 (B6 mice) and 2 (CD40-/- mice) times, respectively, with comparable results. In (***e***) the % specific lysis of 23 respectively 37 bulk CTL cultures derived from B6 mice injected with E1A-peptide in IFA alone (left) or in combination with the anti-CD40 mAb (right) at an E/T of 60 is shown. Mean plus standard deviation of each group are shown (E1A versus E1A+anti-CD40: $p<0.01$, student t-test).

Figure 8: Therapy of HPV16 E6 and E7 transformed cells by combination treatment of peptide together with *in vivo* CD40 triggering

Mice were injected s.c with 25.000 HPV16 transformed syngeneic cells (TC-1). C57BL/6 mice were left untreated (-○-) or after 6 days received 100 μg HPV16 E7-peptide i.p. in IFA (-□-), 100 μg HPV16 E7-peptide i.p. in IFA in combination with the anti-CD40 mAb FGK-45 (-■-) or a control peptide i.p. in IFA in combination with the anti-CD40 mAb FGK-45 (-●-). The percentage of tumor bearing mice is depicted for different treatment groups (n=10) in (***a***). The differences between the group treated with the HPV16-peptide plus the anti-CD40 mAb and the other three groups were statistically significant ($p<0.01$) (Log-Rank test). In (***b***) the percentage of surviving animals is shown (E7-peptide-treated group vs E7-peptide plus anti-CD40-treated group: $p = 0.002$, Log-Rank test).

Making and Using the Invention

The CD40 binding molecules of the invention can be made by conventional production and screening techniques. A rat and a hamster anti-mouse CD40 monoclonal antibody ("Mabs") are each described in *Nature* 393: 474-77 (1998) and are available commercially (Pharmingen, Inc., CA). The anti-mouse CD40 MAb, designated FGK45, which is used in the experiments described below, is described by Rolink. A. *et al., Immunity* 5, 319-330 (1996). Anti-human CD40 MAbs can be made following techniques well-known in the art, and described by G. Köhler and C. Milstein (*Nature*, 1975: 256: 495-497). MAbs can be raised by immunizing rodents (*e.g.* mice, rats, hamsters and guinea pigs) with either native CD40 as expressed on cells or purified from human plasma or urine, or recombinant CD40 or its fragments, expressed in a eukaryotic or prokaryotic system. Other animals can be used for immunization, *e.g.* non-human

primates, transgenic mice expressing human immunoglobulins and severe combined immunodeficient (SCID) mice transplanted with human B lymphocytes. Hybridomas can be generated by conventional procedures by fusing B lymphocytes from the immunized animals with myeloma cells (e.g. Sp2/0 and NS0), as described by G. Köhler and C. Milstein *Id.* In addition, anti-CD40 MAbs can be generated by screening of recombinant single-chain Fv or Fab libraries from human B lymphocytes in phage-display systems. The specificity of the MAbs to CD40 can be tested by enzyme linked immunosorbent assay (ELISA), Western immunoblotting, or other immunochemical techniques. The activating activity of the antibodies on CTLs, in combination with a CTL-activating peptide, can be assessed using the assays described in the Examples below.

For treating humans, the anti-CD40 MAbs would preferably be used as chimeric, Deimmunised, humanized or human antibodies. Such antibodies can reduce immunogenicity and thus avoid human anti-mouse antibody (HAMA) response. It is preferable that the antibody be IgG4, IgG2, or other genetically mutated IgG or IgM which does not augment antibody-dependent cellular cytotoxicity (S.M. Canfield and S.L. Morrison, *J. Exp. Med.*, 1991: 173: 1483-1491) and complement mediated cytolysis (Y.Xu et al., *J. Biol. Chem.*, 1994: 269: 3468-3474; V.L. Pulito et al., *J. Immunol.*, 1996; 156: 2840-2850).

Chimeric antibodies are produced by recombinant processes well known in the art, and have an animal variable region and a human constant region. Humanized antibodies have a greater degree of human peptide sequences than do chimeric antibodies. In a humanized antibody, only the complementarity determining regions

(CDRs) which are responsible for antigen binding and specificity are animal derived and have an amino acid sequence corresponding to the animal antibody, and substantially all of the remaining portions of the molecule (except, in some cases, small portions of the framework regions within the variable region) are human derived and correspond in amino acid sequence to a human antibody. See L. Riechmann et al., *Nature*, 1988; 332: 323-327; G. Winter, *United States Patent* No. 5,225,539; C.Queen et al., U.S. patent number 5,530,101.

Deimmunised antibodies are antibodies in which the T and B cell epitopes have been eliminated, as described in in International Patent Application PCT/GB98/01473. They have reduced immunogenicity when applied *in vivo*.

Human antibodies can be made by several different ways, including by use of human immunoglobulin expression libraries (Stratagene Corp., La Jolla, California) to produce fragments of human antibodies (VH, VL, Fv, Fd, Fab, or $(Fab')_2$, and using these fragments to construct whole human antibodies using techniques similar to those for producing chimeric antibodies. Human antibodies can also be produced in transgenic mice with a human immunoglobulin genome. Such mice are available from Abgenix, Inc., Fremont, California, and Medarex, Inc., Annandale, New Jersey.

One can also create single peptide chain binding molecules in which the heavy and light chain Fv regions are connected. Single chain antibodies ("ScFv") and the method of their construction are described in U.S. Patent No. 4,946,778. Alternatively, Fab can be constructed and expressed by similar means (M.J. Evans et al., *J. Immunol. Meth.*, 1995; 184: 123-138). All of the wholly and partially human antibodies are less

immunogenic than wholly murine MAbs, and the fragments and single chain antibodies are also less immunogenic. All these types of antibodies are therefore less likely to evoke an immune or allergic response. Consequently, they are better suited for *in vivo* adminstration in humans than wholly animal antibodies, especially when repeated or long-term adminstration is necessary. In addition, the smaller size of the antibody fragment may help improve tissue bioavailability, which may be critical for better dose accumulation in acute disease indications, such as tumor treatment.

Based on the molecular structures of the variable regions of the anti-CD40 mAbs or the known CTL-activating peptides, one could use molecular modeling and rational molecular design to generate and screen molecules which mimic the molecular structures of the binding region of the antibodies or the peptides, respectively, and activate CTLs. These small molecules can be peptides, peptidomimetics, oligonucleotides, or other organic compounds. The mimicking molecules can be used for treatment of cancers and infections. Alternatively, one could use large-scale screening procedures commonly used in the field to isolate suitable molecules from libraries of compounds.

The dosage for the molecules of the invention can be readily determined by extrapolation from the *in vitro* tests and assays described below, or from animal experiments or from human clinical trials. The molecules of the invention would be preferentially administered by injection, in the case of antibodies or proteins, although certain small molecules may be suited for oral administration. The assays and tests demonstrating the efficacy of the invention are described below.

Example1: Signaling through CD40 can replace $CD4^+$ helper T cells in CTL priming

A well characterized model system to probe the mechanism of T-cell help for the primary activation of CD8+ CTL responses *in vivo* was used. C57BL/6 (with the major histocompatibility complex (MHC) H-2^b) mice immunized with allogenic BALB/c (H-2^d) mouse embryo cells (MECs) expressing the human adenovirus type 5 early region 1 (Ad5EI-BALB/c MECs) generated strong CTL responses against an H-2D^b-restricted epitope of the adenovirus EIB protein ($EIB_{192-200}$) (Figure 1a). As the allogeneic H-2^d MHC molecules expressed by the Ad5EI-BALC/c MECs cannot prime H-2^b-restricted host CTLs, generation of E1B-specific CTLs must require cross-priming, that is, the uptake and H-2^b-restricted re-presentation of antigen by host APCs. Cross-priming of EIB-specific CTLs is strictly helper-dependent (Figure 1b), as mice depleted of CD4+ T-helper (T_h) cells before immunization no longer mounted an E1B-specific CTL response.

To investigate whether signalling through CD40 can replace $CD4^+$ helper T cells in CTL priming, mice were depleted of $CD4^+$ T cells *in vivo* before immunization with Ad5E1BALB/c MECs. One day after immunization, the mice received a single injection of the activating antibody anti-mouse CD40 mAb FGK45, or of an isotype-matched control antibody. Administration of FGK45 to CD4-depleted, immunized mice resulted in the efficient restoration of E1B-specific CTL responses (Figure 2a) whereas treatment with the control antibody did not (Figure 2b). Priming of E1B-specific CTLs was not detected in naïve mice treated with FGK45 alone (not shown). To address the possibility that the effect of FGK45 was mediated through remaining $D4^+$ cells that were not depleted by treatment with the anti-CD4 antibody, B6 I-A^b knockout mice, which lack mature functional $CD4^+$ peripheral T cells, were immunized with the Ad5EI-BALB/c MECs. The response to immunization in these mice mirrors that seen in the CD4-

depleted mice, in that E1B-specific CTLs were detectable only in mice receiving the CD40-activating antibody (Figure 2c), and not in those receiving the control antibody (Figure 2d).

It was also studied whether the requirement for anti-CD40 antibodies in priming of CTLs in CD4-depleted mice could be replaced by bacterial lipopolysaccharide (LPS) (50 μg intravenous), a potent inducer of proinflammatory cytokines, or by administration of IL-2 (1 x 10^5 units in incomplete Freund adjuvant, subcutaneous) following immunization with Ad5EI-BALB/c MECs. Whereas CD4-depleted mice treated with FGK45 exhibited strong E1B-specific CTL activity, neither LPS or IL-2 treatment resulted in detectable CTL priming (not shown).

Ligation of CD40 on B cells upregulates their costimulatory activity, suggesting a role for these cells in the restoration of CTL priming by treatment with CD40 activating antibodies. To address this question, B6 □MT mice, which lack mature B cells, were immunized with the allogeneic Ad5EI-BALB/c MECs. Cross-priming of E1B-specific CTLs did not require mature B cells (Figure 3a). However, when depleted of $CD4^+$ cells, the B-cell deficient mice did not generate an E1B-specific CTL response (Figure 3b). Activation through CD40 with the FGK45 monoclonal antibody completely restored the capacity of CD4-depleted □MT mice to prime E1B-specific CTLs (Figure 3c). Thus B cells are not required as APCs or accessory cells for cross-priming in this model system, nor are they required for CD40-mediated restoration of cross priming of CTLs in the absence of $CD4^+$ helper T cells. These results demonstrate that activation of bone marrow

derived APC through CD40 can bypass the requirement for $CD4^+$ T-helper cells in the cross-priming of E1B-specific CTLs.

Example 2: Blocking the ability of $CD4^+$ helper T cells to interact with APC through the CD40L-CD40 pathway prevents antigen-specific CTL responses in normal mice

If the CD40L-CD40 interaction represents the physiological pathway used by $CD4^+$ helper T cells to help CTLs, blocking the ability of the $CD4^+$ T cells to interact with APC through CD40L-CD40 interaction would be expected to diminish priming of E1B-specific CTL responses in normal mice. B6 mice were immunized with Ad5E1-BALB/c MECs and then treated with either the CD40L-blocking antibody MR1, or control antibody. Blockade of CD40L results in drastically reduced E1B-specific CTL responses (Figure 4a) compared to the efficient CTL priming seen in mice receiving the control antibodies (Figure 4b). The priming defect induced by CD40L blockade was fully restored following CD40 signalling by FGK45 (Figure 4c). Thus the defect in CTL-priming induced by CD40L blockade lies in the failure of T_H cells to transmit, rather than to receive, CD40L-mediated signals.

Example 3: E1A-specific CTL unresponsiveness after peptide administration

A previously described model system has been used (Toes et al., J. Immunol. 156:3911 (1996)). It has been shown that s.c. vaccination with the Ad5E1A-derived CTL epitope SGPSNTPPEI (SEQ ID NO: 2) in IFA prevents mice from controlling the outgrowth of Ad5E1A-expressing tumors. This indicates that the E1A/IFA vaccine induced suppression rather than induction of E1A-specific CTL immunity. Moreover, administration of the E1A/IFA vaccine to T cell receptor (TCR)-transgenic mice, which

express the TCR α and β chains of an E1A-specific CTL clone, strongly suppressed tumor-specific CTL-mediated immunity. These experiments examined the effects of peptide administration on a monoclonal CTL population. To establish whether s.c. E1A-peptide vaccination also induces E1A-specific CTL tolerance at the polyclonal CTL level, wild type (wt) C57BL/6 mice were injected with either E1A-peptide (Figure 5a and 5b) or a control peptide Figure 5c and 5d). One day later the mice were boosted with a syngeneic cell line expressing high levels of the E1A-peptide at its surface (Figure 5b and 5d). Injection of this cell line into mice primed with the control peptide readily induces E1A-specific immunity (Figure 5d). However, the ability of mice to mount E1A-specific CTL responses was abrogated after injection of the E1A/IFA vaccine (Figure 5b). These data indicate that injection of the E1A-peptide not only leads to E1A-specific tolerance in TCR-transgenic mice but also in mice expressing a polyclonal E1A-specific T cell repertoire.

Since s.c. injection of the E1A/IFA vaccine leads to systemic CTL tolerance, it was investigated whether the E1A-peptide is dispersed systemically and presented to precursor CTL in the periphery. Therefore, mice were injected s.c. with the E1A-peptide or Human Papilloma Virus (HPV) 16 E7-derived control peptide emulsified in IFA. Spleen cells from these mice were isolated 16h later and used as stimulator cells for an E1A-specific CTL clone *in vitro*. Splenocytes from mice injected with the E1A-peptide s.c. induced specific proliferation, whereas splenocytes from mice injected with the E7-peptide s.c. failed to do so (Figure 6). Moreover, a control CTL clone did not proliferate on spleen cells derived from E1A-injected mice (data not shown). Thus, these data

indicate that the E1A-peptide injected s.c. in IFA is systemically presented in the periphery by, amongst others, splenocytes.

In view of the tolerizing effects described above of the E1A-peptide vaccine, there was a question whether CD40-triggering *in vivo* is sufficient to prevent peripheral tolerization of CTL and to restore CTL priming. Therefore, it was investigated whether injection of tolerizing peptides combined with *in vivo* CD40 triggering could prevent the induction of peripheral CTL tolerance leading to tumor-specific CTL immunity.

In Examples 1 and 2 it has been shown that CD40-triggering *in vivo* can replace the requirement for $CD4^+$ T helper cells in priming of helper-dependent CTL responses. Since $CD4^+$ T cell-mediated helper activity has been implicated in the prevention of peripheral CTL tolerance induction, the inventors addressed the question whether CD40-triggering *in vivo* is sufficient to prevent peripheral E1A-specific CTL tolerization. To this end, mice were injected with the E1A/IFA vaccine in combination with the activating anti-CD40 mAb FGK-45. Mice that received this combination mounted strong E1A-specific CTL responses (Figure 7b and 7e), whereas mice that received the E1A/IFA vaccine (Figure 7e) or mAb alone did not (not shown). The combination of E1A/IFA vaccine and anti-CD40 mAb failed to elicit CTL in CD40-deficient mice (Figure 7c and 7d). Furthermore, co-injection of the E1A/IFA vaccine with an isotype-matched control mAb (Figure 6a) or IL-2 failed to convert CTL tolerance induced by the E1A/IFA vaccine into CTL priming (not shown). The range and variation of responses to the E1A-epitope in E1A-peptide only, or E1A-peptide plus anti-CD40-vaccinated animals, is

shown in Figure 7e. Thus, systemic CD40 activation can reverse peptide-induced peripheral CTL tolerance into peptide and tumor-specific CTL mediated immunity.

The induction of E1A-specific immunity strongly correlated with the presence of $CD8^+$ T cells in the spleen of vaccinated mice that stained with PE-conjugated H-2-D^b-tetramers containing the E1A-peptide (D^b/E1A). Within 10 days after vaccination, $CD8^+$ T cells staining with D^b/E1A tetramers could be detected by flow cytometry in mice injected with E1A-peptide and the anti-CD40 mAb, but not in mice injected with E1A-peptide alone (not shown). In the mice injected with E1A-peptide, the percentage of $CD8^+$ cells that stained with the D^b/E1A tetramers was approximately 3%. In mice vaccinated with whole adenovirus, which induces potent E1A-specific immunity, comparable amounts of D^b/E1A tetramer-reactive $CD8^+$ spleen cells were detected. These results indicate that the expansion of E1A-specific $CD8^+$ T cells in mice that received the E1A/IFA vaccine in combination with the anti-CD40 mAb was substantial and equivalent to that found in virus vaccinated animals.

Example 4: CD40-triggering strongly enhances the efficacy of peptide-based anti-cancer vaccines

Although the findings described above show that provision of help through CD40-triggering is sufficient to prevent CTL-tolerization after administration of a tolerogenic peptide-vaccine, they do not address the question whether the efficacy of anti-cancer vaccines that normally induce protective immunity, instead of tolerance, can be enhanced by activation through CD40. It was examined whether CD40-triggering *in vivo* is beneficial to the outcome of vaccination with an HPV16 E7-derived peptide.

Vaccination with this peptide induces protective CTL-mediated immunity against a challenge with HPV16-transformed tumor cells. Moreover, this peptide can be used in a therapeutic setting when loaded on *in vitro* activated DC suggesting that the strength of the anti-tumor response is enhanced when presented by activated DC.

Mice receiving the E7-peptide in combination with CD40-triggering mounted a more potent CTL-response compared to mice treated with E7-peptide only (data not shown), indicating that CD40-triggering also enhances the efficacy of the HPV16 E7-peptide vaccine and confirming the findings with the E1A peptide described above. Moreover, mice treated 6 days after s.c. injection of CD40-negative HPV16 E6/E7 transformed tumor cells with the HPV16 E7-peptide alone (open squares) are able to slow down tumor growth, but eventually most animals succumb to the tumor (Figure 8). When, however, HPV 16 E7-peptide vaccination was combined with injection of the anti-CD40 mAb, tumor growth was markedly reduced and 7 out of 10 mice rejected the tumor, whereas animals injected with a control peptide and the anti-CD40 mAb were unable to control outgrowth of the tumor. These results show that the effect of vaccination regiments can be markedly enhanced when immunization is combined with *in vivo* CD40-triggering. These data provide the basis for the development of extremely potent and novel anti-tumor vaccines for cancer patients.

The foregoing description, terms, expressions and examples are exemplary only and not limiting. The invention includes all equivalents of the foregoing embodiments, both known and unknown. The invention is limited only by the claims which follow and not by any statement in any other portion of this document or in any other source.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a CD40 binding molecule and a CTL activating peptide.

2. The pharmaceutical composition of claim 1 wherein the CD40 binding molecule is an anti-CD40 antibody or a fragment thereof, a peptide, an oligonucleotide or an organic molecule.

3. The pharmaceutical composition of claim 2 wherein the anti-CD40 antibody is human, humanized, chimeric or Deimmunised™.

4. The pharmaceutical composition of claim 1 wherein the CTL activating peptide is the adenovirus-derived E1A peptide, having the sequence SGPSNTPPEI (SEQ ID NO:1), or the HPV16 E7 peptide derived from human papillomavirus type 16, having the sequence RAHYNIVTF (SEQ ID NO:3).

5. A method of treating tumors comprising administering the pharmaceutical composition of any of claims 1 to 4.

6. A method of treating tumors or infectious diseases comprising administering a CD40 binding molecule and a CTL activating peptide.

7. The method of claim 5 wherein the pharmaceutical composition is administered directly to the tumor.

8. A method of treating tumors or infectious diseases comprising administering gene constructs coding for a CD40 binding molecule and a CTL activating peptide.

9. The method of claim 8 wherein the CD40 binding molecule is an anti-CD40 antibody or a fragment thereof, or a peptide, and the CTL activating peptide is peptide is the adenovirus-derived E1A peptide, having the sequence SGPSNTPPEI (SEQ ID NO:2), or the HPV16 E7 peptide derived from human papillomavirus type 16, having the sequence RAHYNIVTF (SEQ ID NO:3).

10. Cells transfected or infected with the gene constructs of claim 8.

11. The method of claims 8 or 9 wherein transfection or infection of the gene constructs is done *ex vivo* or *in vivo*.

12. The method of claim 11 wherein the transfection is done *ex vivo* by electroporation, calcium phosphate transfection, micro-injection or by incorporating the gene constructs into suitable liposomes.

13. The method of claim 12 wherein the infection is done *in vivo* or *ex vivo* by incorporating the gene constructs into a retrovirus, adenovirus or a parvovirus vector, or by incorporating the gene constructs, or the gene constructs with a viral or plasmid vector, into a suitable liposome.

Abstract of the Disclosure

Disclosed is a method and composition for treating tumors or infectious diseases, wherein the composition includes CD40 binding molecules together with CTL-activating peptides, *e.g.*, tumor antigens. Such composition is useful for enhancing the anti-tumor effect of a peptide tumor vaccine, or for otherwise activating CTLs so that the activated CTLs can act against tumorous or infected cells. The CD40 binding molecules can include antibody molecules, as well as homologues, analogues and modified or derived forms thereof, including immunoglobulin fragments like Fab, $(Fab')_2$ and Fv, as well as other molecules including peptides, oligonucleotides, peptidomimetics and organic compounds which bind to CD40 and activate the CTL response.

FIG. 1A

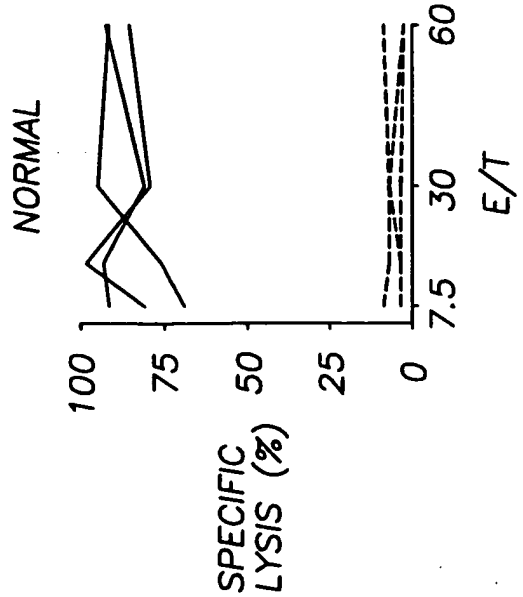

NORMAL
100
75
50
25
0
SPECIFIC LYSIS (%)
7.5
30
60
E/T

FIG. 1B

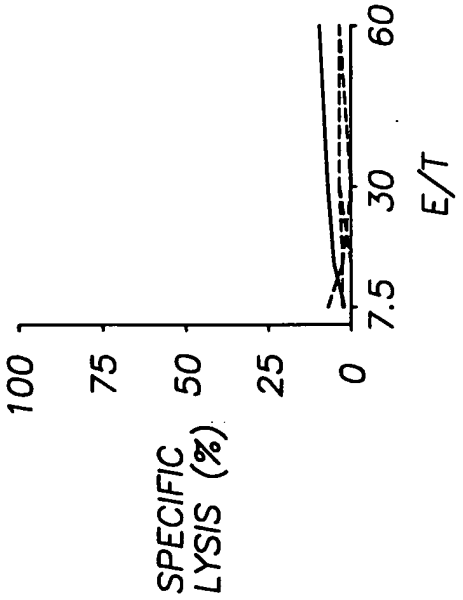

CD4-DEPLETED
100
75
50
25
0
SPECIFIC LYSIS (%)
7.5
30
60
E/T

FIG. 2A

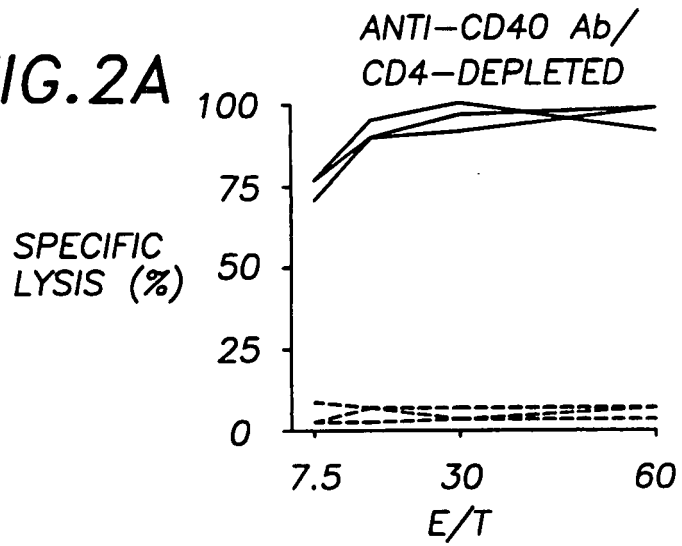


FIG. 2B

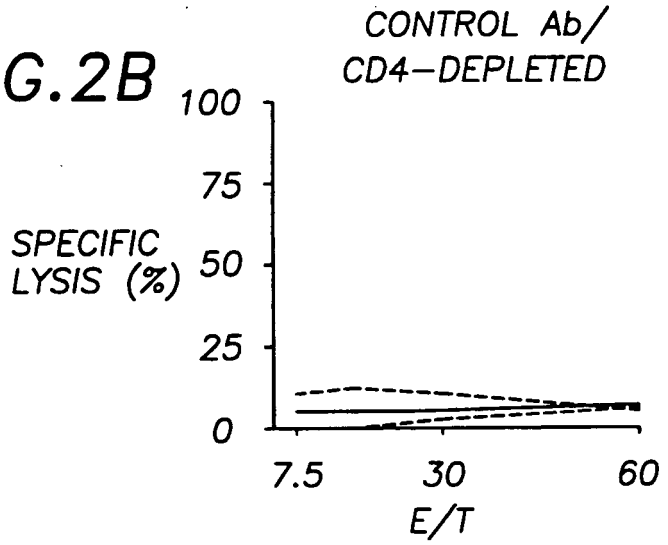


FIG. 2C

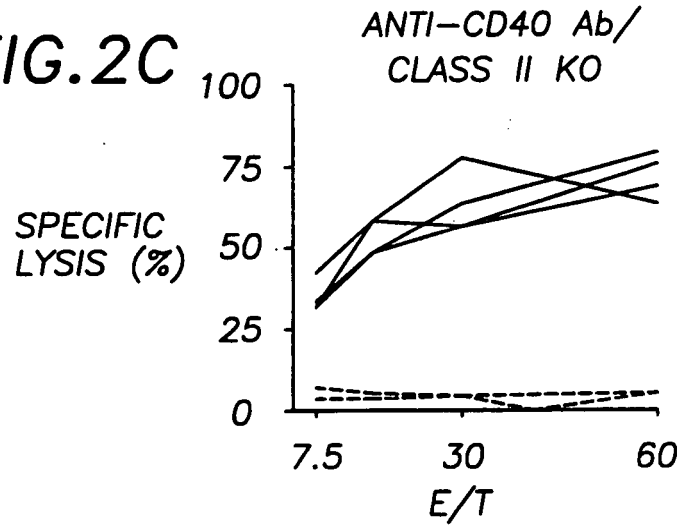


FIG. 2D

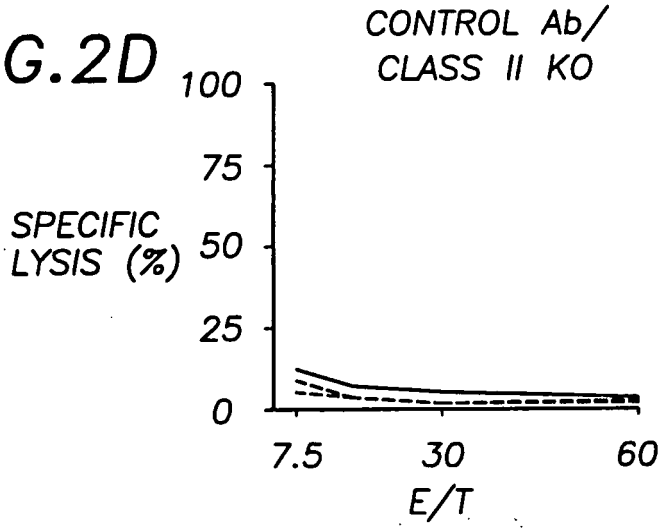


FIG.3A

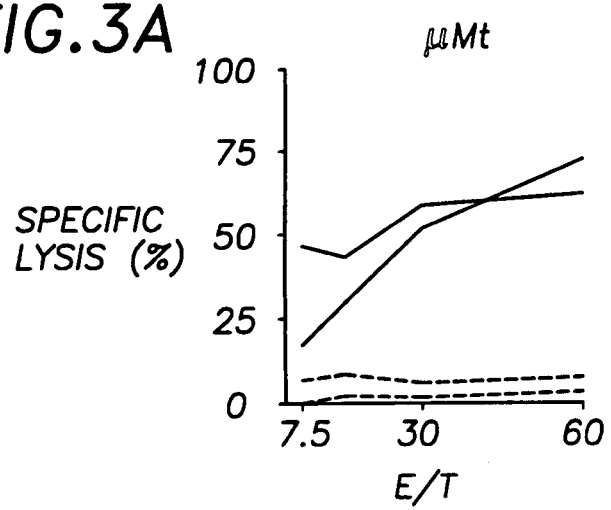


FIG.3B

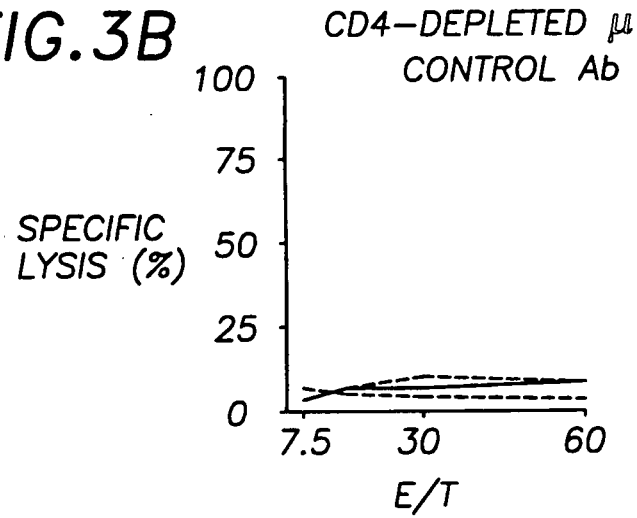


FIG.3C

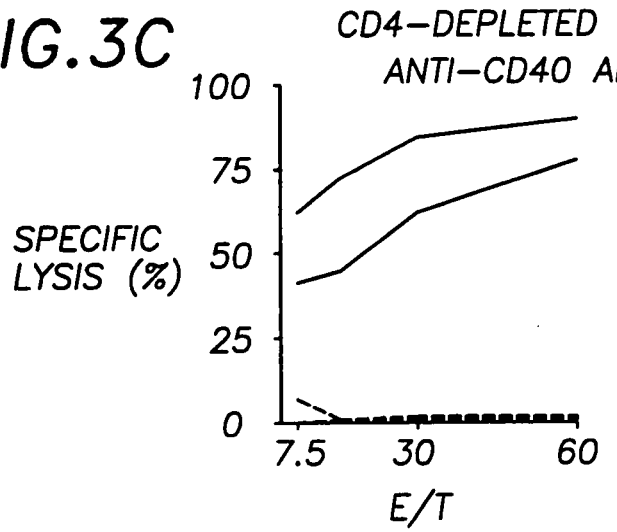


FIG. 4A

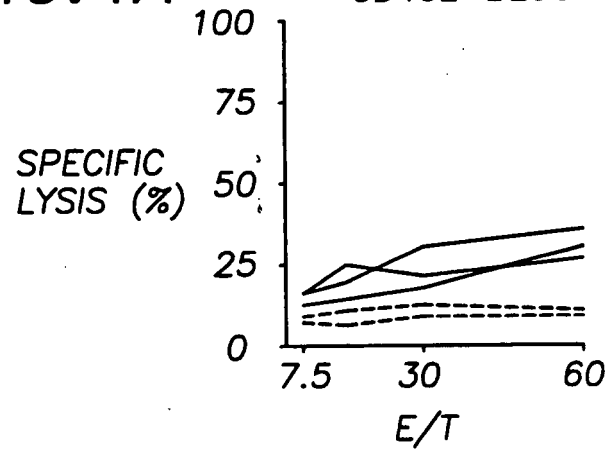


FIG. 4B

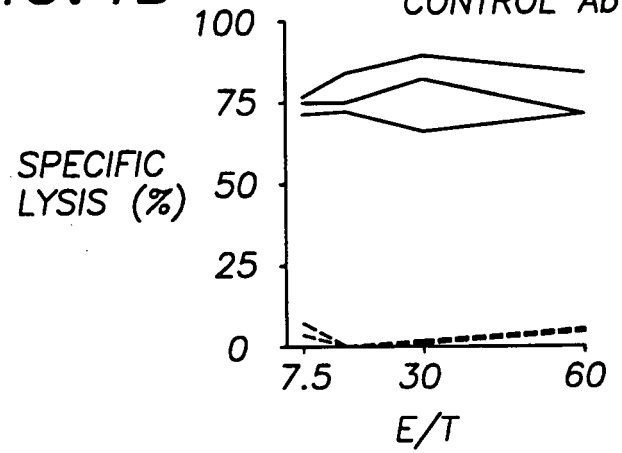


FIG. 4C

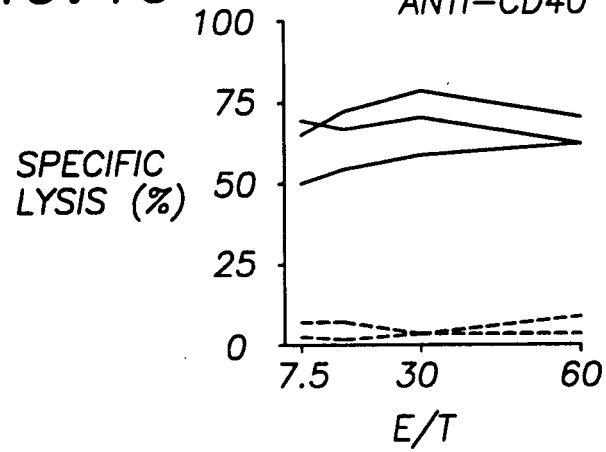


FIG.5A

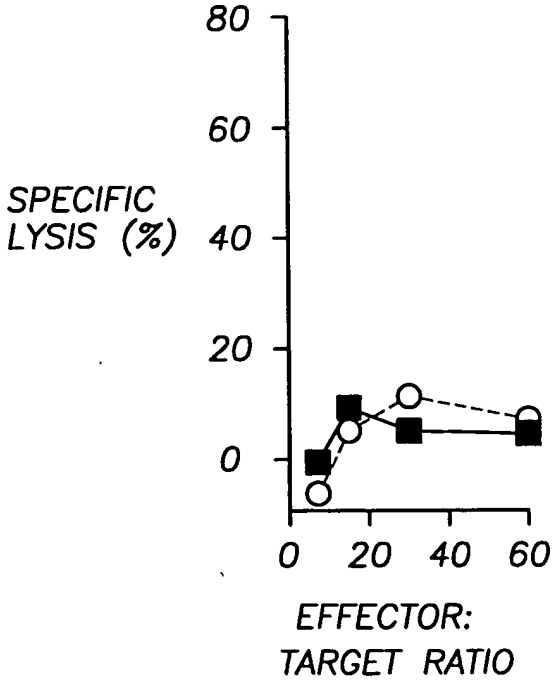


FIG.5B

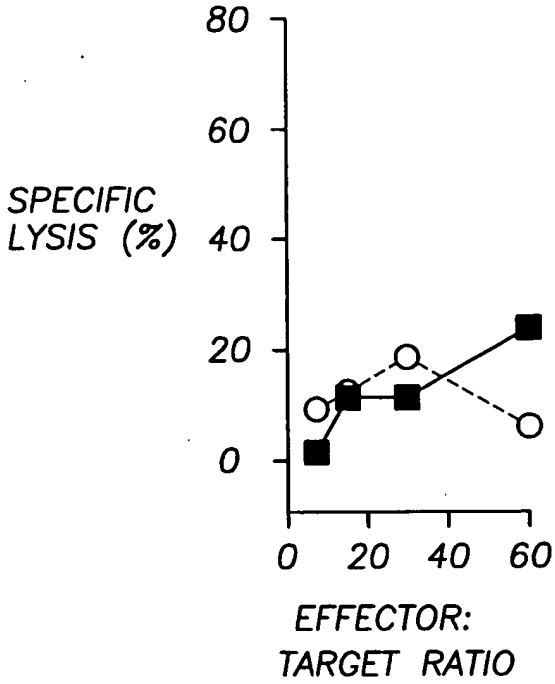


FIG.5C

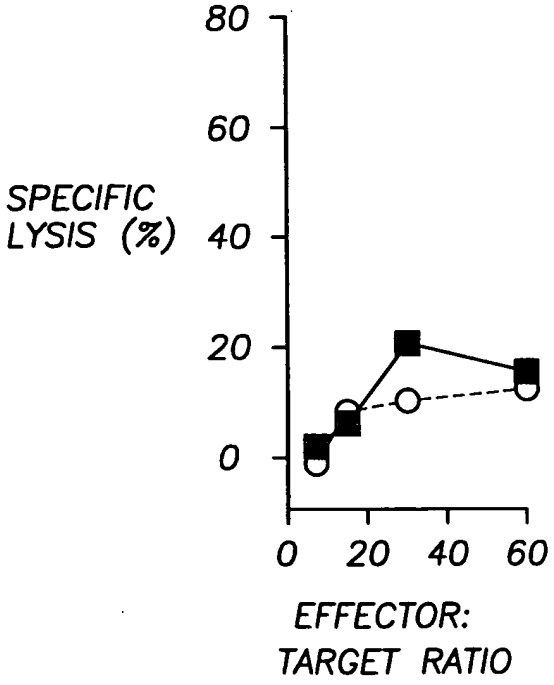
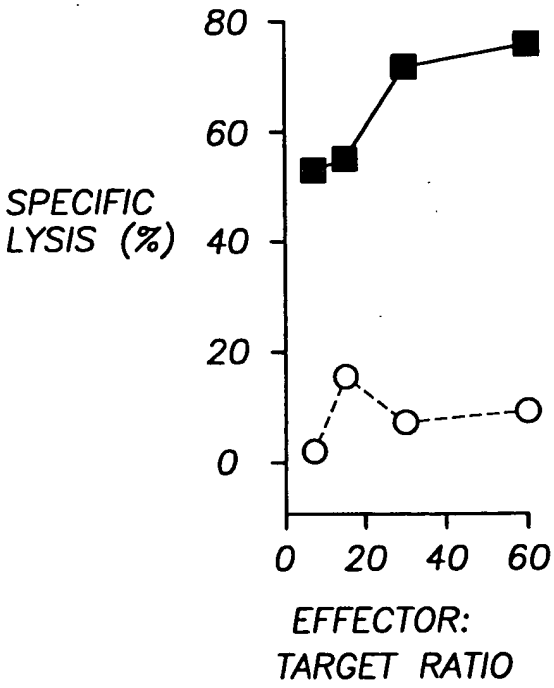


FIG.5D



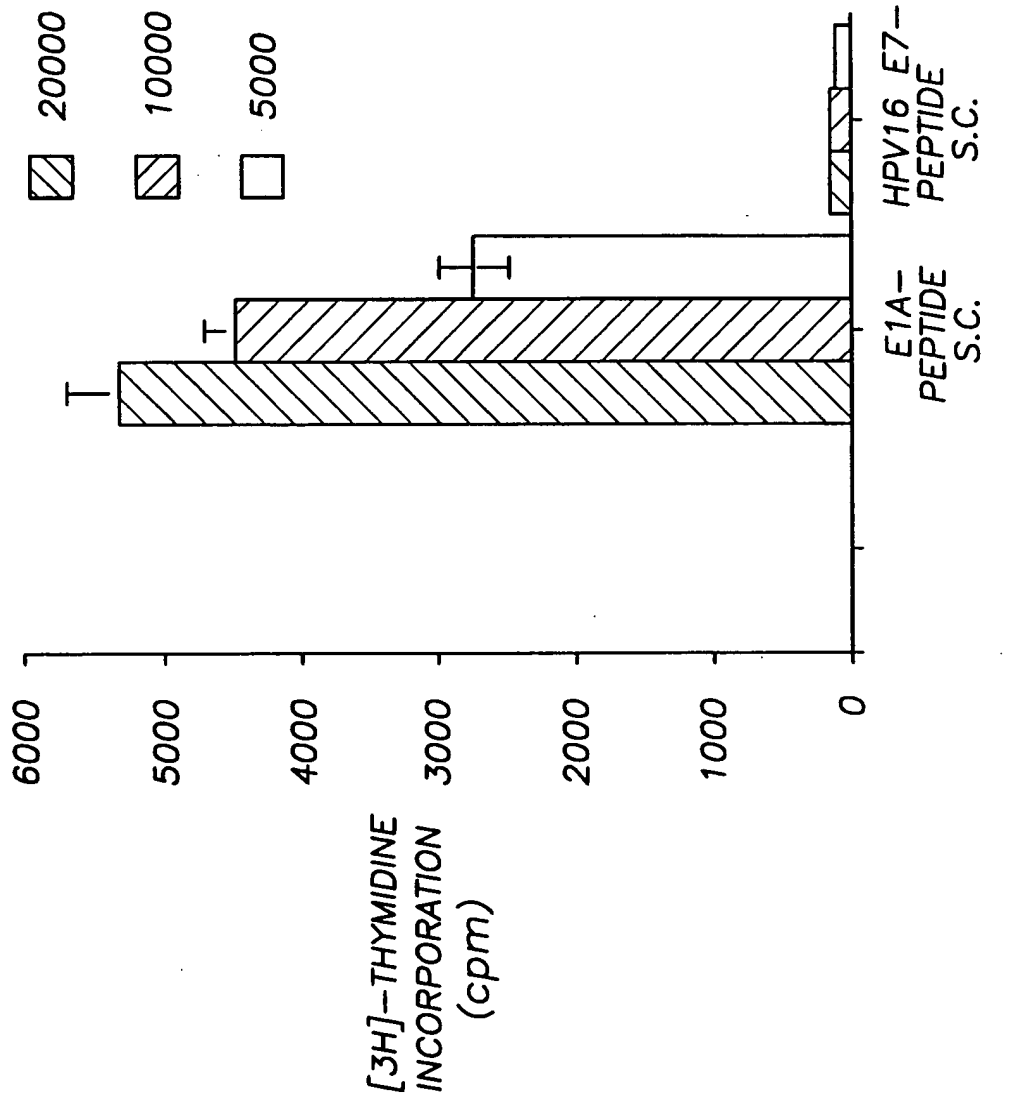

20000
10000
5000
6000
5000
4000
3000
2000
1000
0
[3H]-THYMIDINE INCORPORATION (cpm)
E1A-PEPTIDE S.C.
HPV16 E7-PEPTIDE S.C.

FIG. 6

FIG. 7A

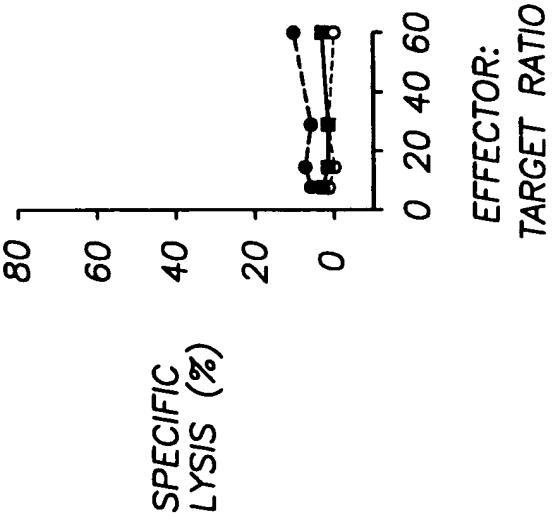

80
60
40
20
0
SPECIFIC LYSIS (%)
0 20 40 60
EFFECTOR: TARGET RATIO

FIG. 7B

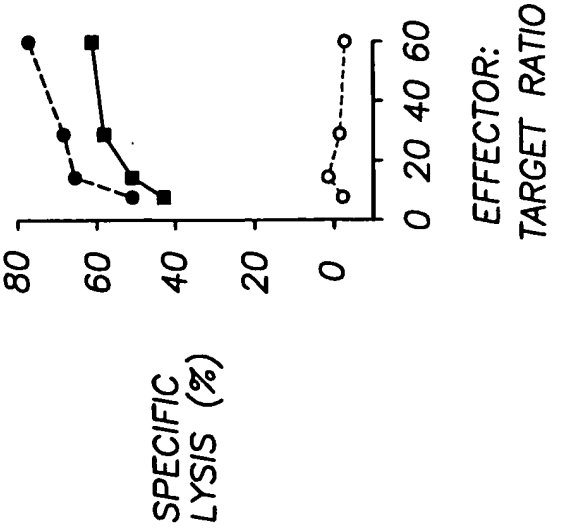

80
60
40
20
0
SPECIFIC LYSIS (%)
0 20 40 60
EFFECTOR: TARGET RATIO

FIG. 7C

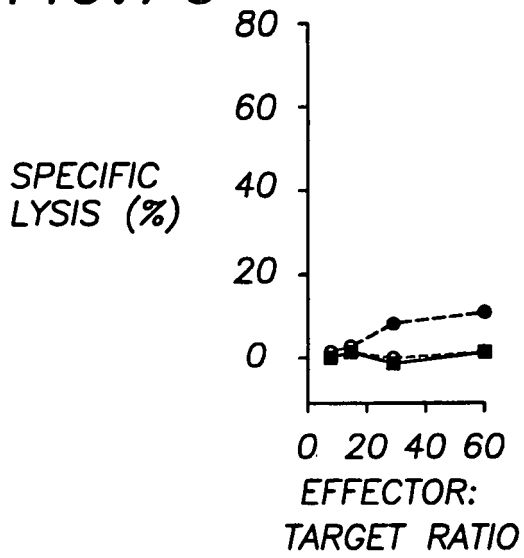


FIG. 7D

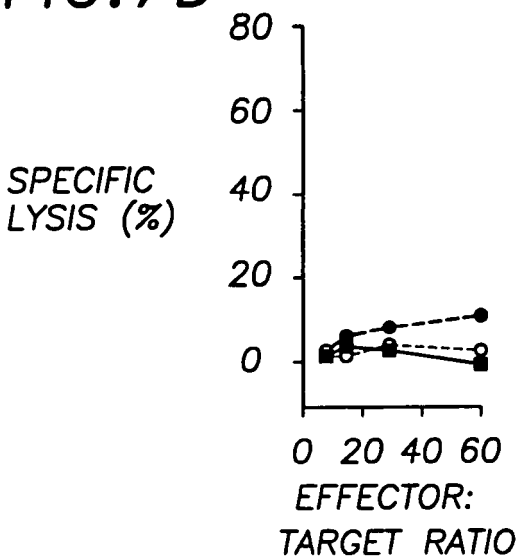


FIG. 7E

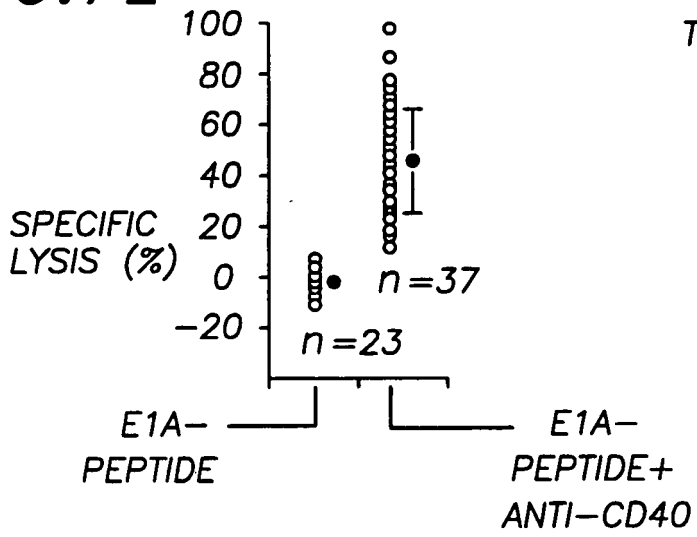


FIG.8A

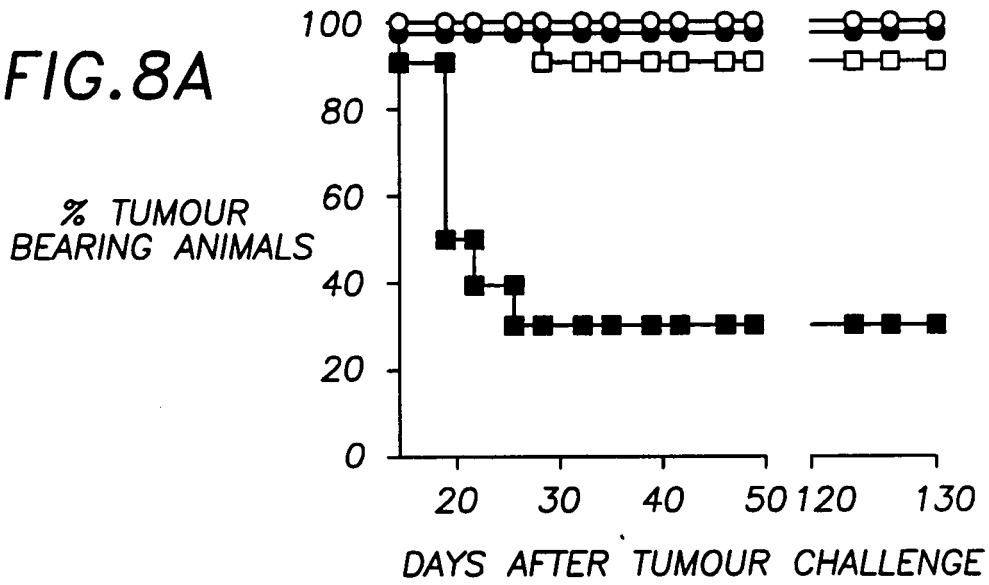
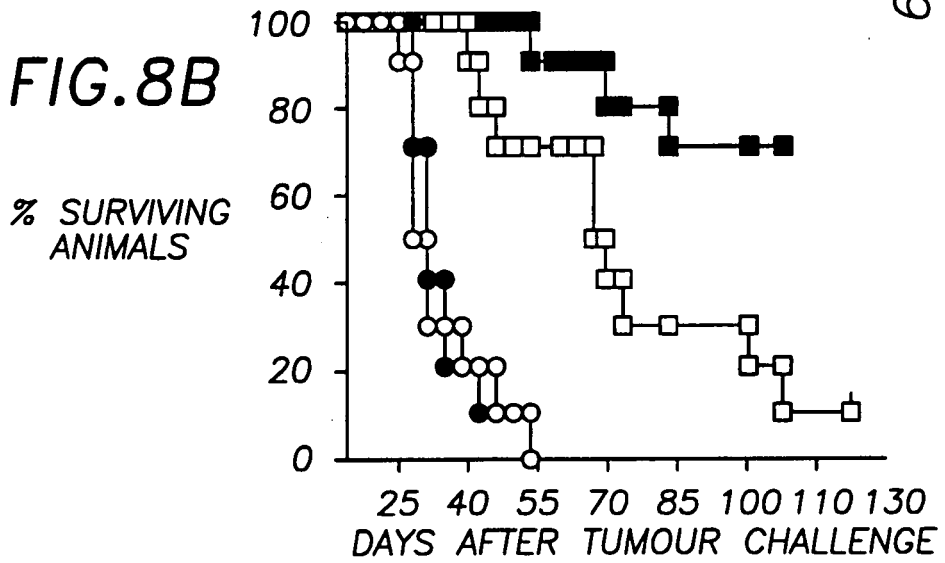

100
80
60
40
20
0
% TUMOUR BEARING ANIMALS
20
30
40
50
120
130
DAYS AFTER TUMOUR CHALLENGE

FIG.8B


100
80
60
40
20
0
% SURVIVING ANIMALS
25
40
55
70
85
100
110
130
DAYS AFTER TUMOUR CHALLENGE